Aims & Objectives of the study

There have been continuous efforts in the development of more efficient immunodiagnostic system, effective vaccine and immunotherapy for the prevention and therapy of HIV/AIDS and AIDS related complexes. But, unfortunately even after two decades of the discovery of HIV, we neither have effective vaccine nor immunotherapeutic agent. Although the diagnostic systems are developed but a continuous move is required to further evolve more and more efficient diagnostic systems.

Although a number of therapeutics are approved for the therapy of HIV, but actually none of the therapeutics is really effective enough to eliminate the infection. Moreover, they cause harmful side effects. Immunotherapy holds the promise of the treatment of unmet diseases. Immunotherapeutics such as specific antibodies bind to and neutralize the target very specifically. Hence, a study on the HIV neutralization effect of bi-specific antibody was aimed which can neutralize protease as well as core protein of HIV-1 and hence reduce the HIV-1 infectivity. Although, a number of antibodies show some efficacy as immunotherapeutics for HIV-1, but in order to improve the therapeutic utility of antibodies next generation of therapeutic antibodies, bi-specific antibody (BsAb), was aimed to be studied.

In order to have an effective preventive vaccine, immunogenic properties and enhancement of immunogenicity of each antigenic epitope was studied.
Abstract of the thesis

There have been continuous efforts in the development of more efficient immunodiagnostic system, effective vaccine and immunotherapy for the prevention and therapy of HIV/AIDS and AIDS related complexes. But, unfortunately after about two decades of the discovery of HIV, we neither have effective vaccine nor immunotherapeutic agent to date. Although the diagnostic systems are developed but a continuous effort is required to further evolve more and more efficient diagnostic systems. In diagnostics, number of different formats have been evolved with different kinds of antigens, i.e. natural, recombinant and synthetic, through the four generations of the development. Synthetic antigens have been found more specific than recombinant antigens which in turn more specific than natural antigens. The synthetic antigenic epitopes, though being more specific and economical, have the limitation of less immunoreactivity on the solid surface due to lesser accessibility to the corresponding antibodies, steric hindrance in antigen-antibodies interactions and shielding of the epitope by blocking agent. Moreover the synthetic p24 antigenic epitopes (both M and O), linear in conformation, which was found less immunoreactive when compared with the native antigenic epitope i.e. recombinant p24 whereas other synthetic epitopes were found immunoreactive to their corresponding antibodies. This study showed that for optimum immunoreactivity, a synthetic epitopes should have the conformation equivalent to the native epitopes, should be highly accessible to the corresponding antibodies, should have minimum steric hindrance and no shielding effect in antigen-antibodies interactions.
In order to enhance the immunoreactivity, synthetic p24 epitopes were conjugated with BSA. The comparative immunoreactivity of the conjugated, free synthetic epitopes and recombinant p24 was studied which showed that the reactivity of conjugated epitope was considerably higher than free epitope and found equivalent to that of recombinant p24. The Western blot non-reactive clinical specimens, which were found reactive with recombinant p24, found nonreactive. This finding showed that by conjugating the p24 epitopes with BSA, it takes the conformation equivalent to native p24 conformation. The extent of the immunoreactivity of synthetic p24 epitopes was further enhanced by conjugating the epitopes with BSA through a decapeptide spacer based on the approach of reducing steric hindrance and shielding effect of blocking agent. The comparative immunoreactivity of the epitopes conjugated through decapeptide spacer to BSA, conjugated to BSA, free synthetic epitopes and recombinant p24 was studied which showed that the immunoreactivity of epitope-spacer-BSA was considerably improved on the immunoreactivity of epitope-BSA. This finding showed that by conjugating the p24 epitopes with BSA through spacer, epitopes are more exposed to their corresponding antibodies with lesser steric hindrance and the shielding effect. Moreover, the delta (δ) value of the assay was also improved considerably using epitope–BSA conjugate which was further improved using spacer in the conjugate.

The findings of p24 epitopes conjugates led to the approach of conjugating the other synthetic epitopes, though they are highly immunoreactive due to their conformation equivalent to the native conformation, with each other to reduce steric hindrance and getting the epitopes more exposed to their antibodies. Eight different sets bivalent
epitopes were produced and compared with their univalent epitopes and found that the bivalent epitopes were having better immunoreactivity than univalent epitopes

A number of antibodies show considerable efficacy as immunotherapeutics. In order to improve the therapeutic utility of antibodies, a bi-specific antibody (BsAb) specific to p24, a structural core protein, and HIV-1 protease was developed and its neutralization effect was studied to reduce HIV infectivity. In this study, p24 was conjugated to HIV-1 protease and injected into the rabbits to develop its antibodies. It was found that p24-Protease conjugate induces immune response to both p24 as well as protease of HIV-1 when immunized with their conjugate. The ELISA titre for p24, protease and p24-Protease conjugate were 200000, 80000 and >200000, respectively. The serum was immunodepleted for the antibodies of p24, HIV-1 Protease and purified the antibodies specific only for the shared epitope of HIV-1 Protease and p24. The studies on the efficacy of the bispecific antibodies to the shared epitopes were carried out by testing the reduction of HIV employing HIV Culture Assay method. The MT4 lymphoblastoid cell line infected with HIV-1 III B 100 TC ID 50 in a 24-well microtiter plate for 14 days. The infectivity level was measured in the supernatants from each culture well by assaying HIV p24 antigen. The p24 and protease shared antibodies reduced the progression of HIV-1 infection by ~ 80 % whereas anti p24 antibodies reduced the progression of HIV-1 infection by ~ 60 %, anti protease antibodies reduced the progression of HIV-1 infection by ~ 60 %, The antiserum to P24-Protease reduced the progression of HIV-1 infection by ~ 70 %, the anti p24 antibodies depleted serum reduced the progression of
HIV-1 infection by ~70% and anti p24 and anti protease antibodies depleted serum reduced the progression of HIV-1 infection by ~70%.

The development of an effective vaccine is still a question mark to the HIV researchers, I studied the efficacy of the antigenic epitopes in immunogenecity and persistency of the immune response. Since the epitopes were hapten and could not elicit the immune response. They were targeted to the immune system on the surface of the multilamellar liposomes. The epitopes were incorporated onto the liposome membrane surface by conjugating them with phosphatidylethanolamine and incorporation of the epitopes – PE conjugates in the formulation of liposomes. In addition, the liposome were sterically protected by an amphiphilic derivative of poly(ethylene glycol) (PEG) or a PEG-like polymer (mPEG20 kD). It was found that antigenic epitopes carried to the immune system on the surface of liposomes, conjugated with PEG, showed very high immune response and the antibody titre was also found long lasting compared to free epitopes as well as epitope-liposome conjugates.

For development of efficient diagnostic system, three bivalent epitopes from the immunodominant domains of envelop region of HIV were selected based on their immunoreactivity. These bivalent epitopes not only covers the detection of HIV of subtype ‘M’ but also subtype ‘O’. Since antibodies to these epitopes last till the infection persists, there was no need to include the epitopes from other domains. Since, antibodies to p24 appear first after seroconversion, the epitopes of p24 from both the subtypes (‘M’ as well as ‘O’) conjugated with BSA through spacer were also selected to
reduce the window period of diagnosis. All the parameters of the assay were optimized for the modified epitopes. The performance of this assay was compared with that of US FDA approved tests and found comparable. Amongst all the reagents in ELISA, anti hIgG-HRP conjugate, solid phase immobilized epitopes and the enzyme substrate-chromogen (TMB-H₂O₂) are very critical and hence an effort was put to stabilize them. They all were stabilized in liquid state and in ready to use form.